REVIEW SUMMARY

CELLULAR BIOPHYSICS

Liquid phase condensation in cell physiology and disease

Yongdae Shin and Clifford P. Brangwynne*

BACKGROUND: Living cells contain distinct subcompartments to facilitate spatiotemporal regulation of biological reactions. In addition to canonical membrane-bound organelles such as secretory vesicles and endoplasmic reticulum, there are many organelles that do not have an enclosing membrane yet remain coherent structures that can compartmentalize and concentrate specific sets of molecules. Examples include assemblies in the nucleus such as the nucleolus, Cajal bodies, and nuclear speckles and also cytoplasmic structures such as stress granules, P-bodies, and germ granules. These structures play diverse roles in various biological processes and are also increasingly implicated in protein aggregation diseases.

ADVANCES: A number of studies have shown that membrane-less assemblies exhibit remarkable liquid-like features. As with conventional liquids, they typically adopt round morphologies and coalesce into a single droplet upon contact with one another and also wet intracellular surfaces such as the nuclear envelope. Moreover, component molecules exhibit dynamic exchange with the surrounding nucleoplasm and cytoplasm. These findings together suggest that these structures represent liquid-phase condensates, which form via a biologically regulated (liquid-liquid) phase separation process. Liquid phase condensation increasingly appears to be a fundamental mechanism for organizing intracellular space. Consistent with this concept, several membrane-less organelles have been shown to exhibit a concentration threshold for assembly, a hallmark of phase separation. At the molecular level, weak, transient interactions between molecules with multivalent domains or intrinsically disordered regions (IDRs) are a driving force for phase separation. In cells, condensation of liquid-phase assemblies can be regulated by active processes, including transcription and various posttranslational modifications. The simplest physical picture of a homogeneous liquid phase is often not enough to capture the full complexity of intracellular condensates, which frequently exhibit heterogeneous multilayered structures with partially solid-like characters. However, recent studies have shown that multiple distinct liquid phases can coexist and give rise to richly structured droplet architectures determined by the relative liquid surface tensions. Moreover, solid-like phases can emerge from metastable liquid condensates via multiple routes of potentially both kinetic and thermodynamic origins, which has important implications for the role of intracellular liquids in protein aggregation pathologies.

OUTLOOK: The list of intracellular assemblies driven by liquid phase condensation is growing rapidly, but our understanding of their sequence-encoded biological function and dysfunction lags behind. Moreover, unlike equilibrium phases of nonliving matter, living cells are far from equilibrium, with intracellular condensates subject to various posttranslational regulation and other adenosine triphosphate–dependent biological activity. Efforts using in vitro reconstitution, combined with traditional cell biology approaches and quantitative biophysical tools, are required to elucidate how such nonequilibrium features of living cells control intracellular phase behavior. The functional consequences of forming liquid condensates are likely multifaceted and may include facilitated reaction, sequestration of specific factors, and organization of associated intracellular structures. Liquid phase condensation is particularly interesting in the nucleus, given the growing interest in the impact of nuclear phase behavior on the flow of genetic information; nuclear condensates range from micrometer-sized bodies such as the nucleolus to submicrometer structures such as transcriptional assemblies, all of which directly interact with and regulate the genome. Deepening our understanding of these intracellular states of matter not only will shed light on the basic biology of cellular organization but also may enable therapeutic intervention in protein aggregation disease by targeting intracellular phase behavior. ■

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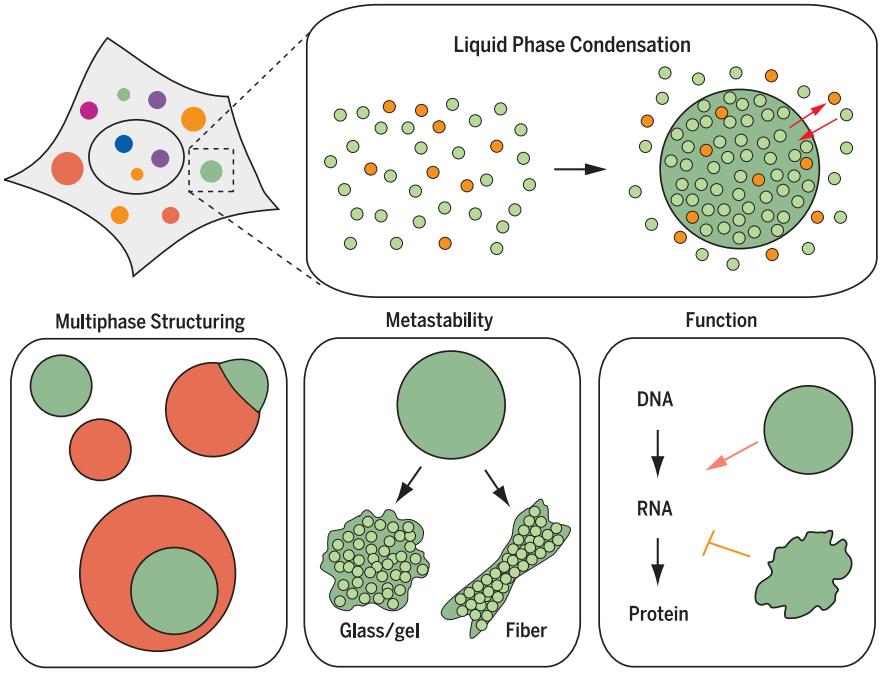

Liquid phase condensation: An emerging paradigm of cellular organization. Living cells contain various types of condensed liquid-like structures enriched with a distinct set of biomolecules that assemble through regulated phase separation. Sequence-encoded physicochemical properties lead to rich intracellular phase behaviors, including multiphase structuring and emergence of solid-like states from metastable liquids. Liquid condensates affect the flow of information in the cell, often through affecting RNA transcription and protein translation.

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Liquid phase condensation in cell physiology and disease

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Phase transitions are ubiquitous in nonliving matter, and recent discoveries have shown that they also play a key role within living cells. Intracellular liquid-liquid phase separation is thought to drive the formation of condensed liquid-like droplets of protein, RNA, and other biomolecules, which form in the absence of a delimiting membrane. Recent studies have elucidated many aspects of the molecular interactions underlying the formation of these remarkable and ubiquitous droplets and the way in which such interactions dictate their material properties, composition, and phase behavior. Here, we review these exciting developments and highlight key remaining challenges, particularly the ability of liquid condensates to both facilitate and respond to biological function and how their metastability may underlie devastating protein aggregation diseases.

A central aspect of biological organization is the compartmentalization of biological systems. Cells are faced with the daunting task of spatiotemporally organizing thousands of simultaneous molecular reactions, which must be coordinated for proper function. To achieve this, the cellular interior is subdivided into dozens of different types of membrane-bound organelles, from the sequestering of genetic material into the nucleus to the more richly shaped Golgi apparatus and endoplasmic reticulum. These and other vesicle-like organelles provide our textbook understanding of intracellular organization, arising from the amphiphilic nature of phospholipids that define their membrane boundaries. Indeed, the term "organelle" is often mistaken as being synonymous with phospholipid-defined compartments.

Liquid droplets in the cell

Despite the dominance of this view of intracellular compartmentalization through canonical vesicle-like organelles, some of the first organelles to be discovered are actually outside of this paradigm. These include the nucleolus (*1*) and Cajal bodies (*2*), both of which we now understand are not membrane-delimited structures but rather represent "open" macromolecular assemblies. These organelles, held together via weak interactions between components, are not structurally defined macromolecular complexes, such as the ribosome. Instead, they reflect collections of thousands of molecules whose relative organization is typically highly dynamic. Advances in modern cell biology and microscopy have revealed a plethora of such intracellular condensates. In many cases, these structures are composed of both protein and RNA molecules and are known as ribonucleoprotein (RNP) bodies/granules (Box 1) (*3*–*5*). These include nuclear bodies such as Cajal bodies, nucleoli, and nuclear speckles and also many cytoplasmic structures, including P granules, stress granules, and processing bodies (P-bodies). Other membraneless bodies are involved in cell signaling—for example, cytoplasmic Dvl clusters seen in Wnt signaling (*6*, *7*) as well as signaling clusters in T cell activation (*8*). Despite their dynamic nature, many of these condensates can maintain their overall size and shape for minutes or hours while exchanging their components with the surrounding cytoplasm or nucleoplasm on time scales of seconds (*9*–*11*). As we discuss below, studies on germline P granules in *Caenorhabditis elegans* have shown that these structures behave as liquid droplets, in that they are round and, upon contact with one another, coalesce into a single larger spherical assembly (Fig. 1) (*12*). Moreover, they can exhibit apparent "wetting" behavior when in contact with surfaces such as the nuclear envelope, resembling water droplets beading up on a hydrophobic surface such as a freshly waxed car. Nucleoli exhibit similar liquid-like behavior (Fig. 1) (*13*), and such dynamic fluid properties have been increasingly observed for both native intracellular condensates (*14*, *15*) and synthetic ones (*16*–*18*), with some important variations, as we discuss below. Although precise measurement of their biophysical properties is challenging because of the small size scales of these intracellular structures (typically ~1 μm diameter), combined in vitro and in vivo studies using a variety of techniques have revealed quantitative signatures of macromolecular liquids, including viscous stress relaxation and low surface tensions. These findings all indicate that many of these structures represent condensed liquid-like states of intracellular matter (Fig. 1).

Phase transitions

These diverse liquid-like droplets of biomolecules, which self-assemble within another liquid—the cytoplasm or nucleoplasm—are increasingly recognized as arising from a physicochemical process known as liquid-liquid phase separation, sometimes also called coacervation. Phase-separated liquids are familiar from everyday experience with immiscible fluids such as water and oil, which will always "demix" and phase separate, no matter how vigorously shaken. Also commonly observed are phase transitions of water itself, which can exist in solid, liquid, or gas phases. For example, high concentrations of gaseous water (high humidity) and low temperatures can promote condensation into water droplets, such as the dewdrops seen condensing on blades of grass. The liquid state is similar to the gaseous state, in that molecules are unorganized and very mobile, but in the liquid state, they are in a highly condensed droplet form. Upon further lowering the temperature, water molecules can snap into register, forming solid crystalline ice. In each of these three states, the water molecules themselves are chemically the same, but their higher-order organization is completely different, with dramatic consequences that are readily visible on the macroscale.

Phase transitions are not restricted to small molecules such as water, and it has long been recognized that biomolecules can undergo similar transitions. For example, protein solutions are known to form crystalline assemblies under

Box 1. Nomenclature.

A wide variety of names have been used for the structures we consider here. When they are RNA- and protein-rich, they are variously referred to as "RNA granules" (*4*), "RNP bodies" (*163*), or "RNP droplets" (*47*). When referring to nuclear structures, these are typically referred to as "nuclear bodies" (*3*). Moreover, membraneless assemblies associated with signaling are often referred to as "signaling clusters" (*164*). In oral presentations, the general term "phases" is increasingly used, which is potentially confusing given its use in relation to phases of the cell cycle. Many other names have also been used, including "assemblages" (*165*), "pleiomorphic ensembles" (*166*), and "membraneless organelles" (*167*, *168*). This nomenclature expansion is summarized by the famous quip "scientists would rather use each other's toothbrushes than each other's terminology" (*169*). Given an ever increasing number of studies suggesting that these structures arise from a form of liquid phase condensation, as well as historical (*23*) and more recent (*170*) uses, we will use the term "condensate" as a simple and general term for any membraneless intracellular assembly.

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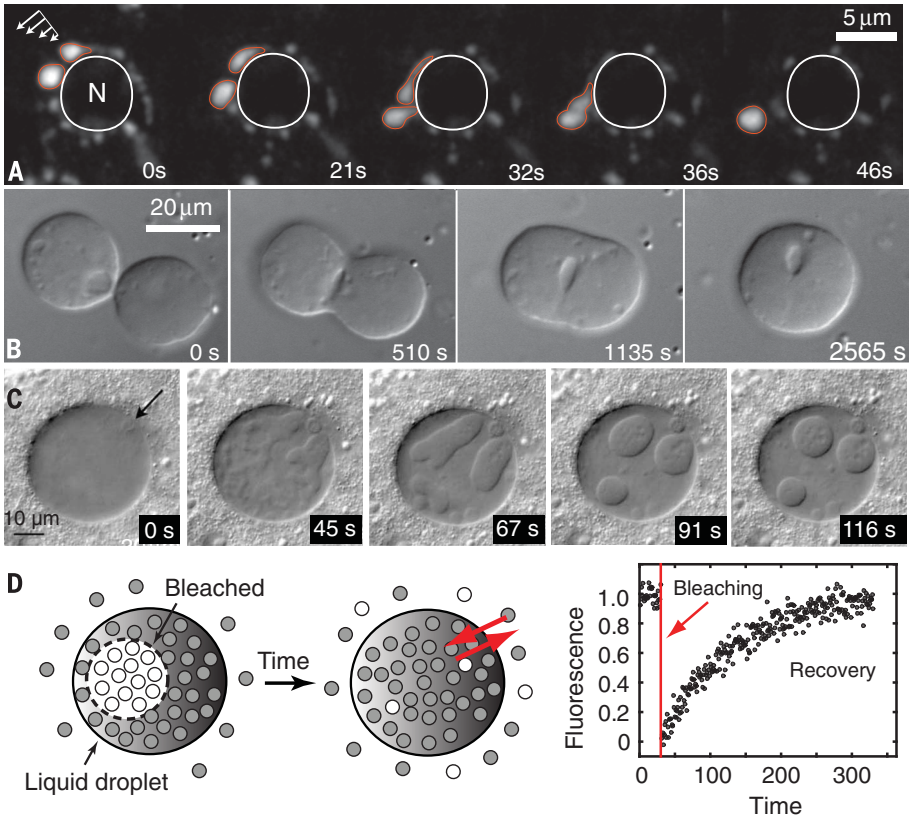

Fig. 1. Liquid phase behaviors of various droplet condensates. (**A**) P granules flow, coalesce, and drip off the surface of a nucleus in response to shear (*12*). (**B**) Spherical nucleoli from *Xenopus laevis* germinal vesicles coalesce into a larger sphere (*13*). (**C**) Liquid-like nuclear bodies induced to nucleate and grow in the nucleus of a *Drosophila* oocyte (*160*). (**D**) Molecules within a liquid phase exhibit dynamic reorganization, which can be probed with FRAP.

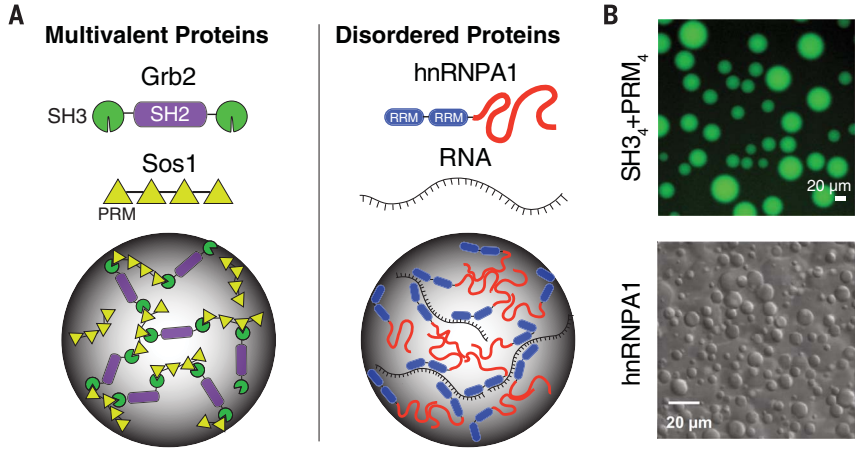

Fig. 2. Molecular features driving intracellular phase separation. (**A**) Weak transient interactions between multivalent proteins drive liquid-liquid phase separation. Regulatory proteins in many signal transduction pathways often contain a set of protein-interaction domains, including SH3 and PRM, as shown here. Proteins harboring IDRs are also key for driving liquid-liquid phase separation. Many RNA-binding proteins have modular architectures in which different sequences of IDRs are appended to various RNA-binding domains, including RRM, RGG repeats, and enzymatic domains. IDRs alone can drive phase separation, but RNA can further promote this process by interacting through RNA-binding domains. (**B**) (Top) A mixture of two model proteins, repeats of SH3 and PRM, undergoes liquid-liquid phase separation in vitro (*17*). (Bottom) The purified hnRNPA1, an RNA-binding protein with a C-terminal IDR, also forms liquid droplets (*15*).

different buffer conditions, and coaxing proteins to crystallize is an essential step in solving the structure of folded proteins with x-ray crystallography (*19*, *20*). In crystallizing into a solid state, protein solutions can also form condensed liquid droplets and are often observed before the appearance of crystals (*21*, *22*). Protein liquids have also been of interest owing to several lines of evidence that suggest liquid phase condensation of proteins within the lens of the eye underlies cataract formation (*23*).

Despite the long-standing interest in protein phase behavior among structural biologists, only recently has it become clear that phase transitions play a ubiquitous role in organizing the contents of living cells (Box 2). These types of phase transitions typically occur above a saturation concentration, the concentration threshold above which two distinct phases demix, resulting in one phase highly concentrated in a given set of molecules and one phase relatively more dilute. Consistent with this model, several condensates, including nucleoli (*24*) and stress granules (*15*), have been shown to assemble only above a concentration threshold. Moreover, the overexpression of key protein components is sufficient to drive stress granule assembly, even in the absence of any stress (*25*–*27*). The molecular features underlying concentration-dependent intracellular phase transitions are only beginning to be understood.

Proteins driving phase separation

Phase transitions represent thermodynamic processes in which a system tends to go toward the lowest-energy state, which is determined by a combination of entropic as well as enthalpic contributions (*28*, *29*) (Box 3). In general, entropy tends to keep a system well mixed; entropy should counteract phase separation because there are more ways to configure a well-mixed system. This is why increasing temperature causes water droplets to dissolve. Thus, the system is usually required to have some attractive interactions between molecules to drive them to phase-separate into a condensed state. As we discuss below, in living cells these interactions are mediated largely by the cell's biopolymers, particularly protein, RNA, and DNA. From decades of theoretical and experimental work on phase transitions in nonliving polymeric systems, it is known that one must also take into account the configurational entropy of the individual chain, which effectively lowers the energetic contribution from mixing entropy because of connectivity of individual subunits (*28*, *30*). In practice, this means that polymeric solutions are more prone to undergo phase separation, which has important biological implications.

Multivalency

A key molecular driving force underlying the assembly of liquid condensates is multivalent protein-protein (and protein-RNA) interactions (Fig. 2). Indeed, many proteins exhibit several repeats of the same type of domain, or different types of modular interaction domains (*31*, *32*).

Box 2. A brief history of cytoplasmic liquid phases.

Studies on the physical nature of the cytoplasm have a long history. In the early 1900s, interdisciplinary biologists including Edmund B. Wilson, Ernest E. Just, and Frank Lillie viewed the cytoplasm as a special type of active fluid. In 1899, Wilson published an article in *Science* on the "Structure of protoplasm," in which he asserted that "The living protoplasm ... is a liquid, or rather a mixture of liquids, in the form of a fine emulsion consisting of a continuous substance in which are suspended drops ... of different chemical nature" (*171*). Over the intervening decades, phase transitions have been proposed to play a role in various aspects of cell organization, most notably in the lipid raft model, which views the two-dimensional (2D) organization of lipid membranes as arising from liquid-liquid phase separation of cholesterol, lipid, and various membrane-associated proteins (*172*). Many researchers had also speculated that phase separation may be similarly relevant within the 3D cell interior (*173*–*175*) and could potentially underlie various "protein condensation diseases" (*23*). Recent discoveries on the liquid phase nature of P granules (*12*), nucleoli (*13*, *64*, *65*), and dozens of other such fluid condensates have revealed that phase transitions are key for organizing the contents of living cells. Together with a rapidly advancing understanding of the molecular driving forces underlying intracellular liquid-liquid phase separation, this work has ushered in an exciting new era in the long history of physicochemical studies of cytoplasm.

Among others, these include repeats of the SH3 domain, which binds to proline-rich motifs (PRMs), both of which are found in many proteins, often forming repetitive modules that are thought to be important for signaling complexes. The importance of these repetitive domains in driving phase separation has been elucidated by using synthetic repeats of the SH3/PRM system (*17*). It was found that by making protein constructs that consist of repeats of three or more SH3 domains, $(SH3)_n$, and mixing with similarly repeating PRM domains, $(PRM)_n$, liquid droplets form. This was particularly true for high-valency constructs ($n \geq 3$), and the module concentration at which droplets are observed decreases with higher valency. Moreover, when such complementary pairs of constructs are expressed within living cells, they are observed to form liquid-like condensates, apparently undergoing phase separation.

Given the ability of a small subset of proteins to drive phase separation, how might these proteins recruit other molecules into the droplet? Important insights have come from recent work using repetitive domains of the binding partners small ubiquitin-like modifier (SUMO) and the SUMO interacting motif (SIM) (*33*), commonly found in various condensates, including nuclear promyelocytic leukemia (PML) bodies (*34*). Like the SH3/PRM system, when higher-valency constructs are mixed in vitro, they undergo liquid-liquid phase separation, forming a droplet comprising a concentrated $(SUMO)_n$ and $(SIM)_m$ "scaffold." When "client" species of either monomeric SUMO or monomeric SIM are introduced, in some cases the client species partition into droplets, whereas sometimes they do not. If $n > m$, then there is a higher concentration of SUMO scaffolds with unbound free sites within droplets, resulting in strong recruitment of SIM client molecules. Conversely, when $n < m$, SUMO client molecules are strongly recruited. Important challenges remain to extend the results of this simple model system so as to understand the full complexity of physiological condensates. But these results show that the relative stoichiometry and valency of interacting molecules can play key roles in compositional control.

Intrinsically disordered protein regions

Many intracellular condensates, particularly RNP bodies, are highly enriched in proteins that contain stretches of low sequence complexity, which often exhibit conformational heterogeneity (Fig. 2) (*35*–*38*). These so-called intrinsically disordered proteins/regions (IDPs/IDRs) are typically enriched in particular polar and charged amino acids, including glycine (G), serine (S), glutamine (Q), proline (P), glutamic acid (E), lysine (K), and arginine (R) (*39*, *40*). Although IDRs generally lack hydrophobic residues that drive high-order folding, the sequences found in condensates are nonetheless often interspersed with aromatic residues, particularly tyrosine (Y) and phenylalanine (F). These residues pattern the polypeptide backbone to enable charge-charge, charge-π, and π-π stacking interactions (*30*). One well-studied example is the germ granule protein DDX4, a RNA helicase with IDRs at both its N and C terminus (*16*). When expressed and purified in vitro, the N-terminal IDR alone is sufficient for phase-separating into liquid droplets, whose formation is promoted by low temperatures and low salt concentrations. Moreover, when a yellow fluorescent protein–tagged DDX4 IDR construct is expressed in cells, it forms droplets within the cell nucleus. Remarkably, as with in vitro droplets, these intracellular DDX4 condensates are highly sensitive to temperature changes, dissolving at high temperatures and recondensing at lower temperatures.

The closely related RNA helicase LAF-1 forms similar droplets in vitro and contributes to P granule assembly (*41*). Moreover, LAF-1 droplets also dissolve at high salt concentration, underscoring the role of charge-mediated interactions. The IDRs within DDX4 and LAF-1 are polyampholytic; they contain a mix of positively and negatively charged amino acids. The way charges pattern, not just the presence of charges, has been shown to be important in promoting phase separation (*16*, *42*). However, the mechanism underlying charge pattern–driven phase separation is still poorly understood. Atomic simulations suggest that the conformational properties, which are determined by the balance of intrachain electrostatic interactions versus chain-solvent interactions, are highly dependent on the degree of charge segregation along the chain (*43*–*45*). Moreover, LAF-1 IDR simulations reveal particularly large conformational fluctuations, with a large volume sampled by the chain, which in turn enables formation of liquid droplets with a high permeability and low protein density (*46*); indeed, many intracellular RNP condensates appear to exhibit low density (*47*, *48*). Thus, information encoded in the protein sequence appears to affect conformational fluctuations and associated intermolecular interactions, which in turn dictates the biophysical properties of the emergent phases. Recent efforts have begun to systematically evaluate the link between varied protein sequences and phase behavior (*49*), but more work is needed.

A variety of other IDR sequences have recently been shown to drive liquid-liquid phase separation in vitro, including the amyotrophic lateral sclerosis (ALS)–related and stress granule–associated proteins hnRNPA1, FUS, and TDP43 (*14*, *15*, *50*–*52*); the mitotic spindle protein BuGZ (*53*); and the Q-rich cell-cycle regulatory protein Whi3 (*54*). But in some cases, their phase separation may involve distinct physicochemical driving forces. For example, FUS, in contrast to DDX4 and LAF-1, harbors mostly polar and aromatic residues but lacks charged residues, and FUS IDR exhibits liquid-liquid phase separation preferentially at higher salt concentrations (*50*); additional studies on Hofmeister salts may help elucidate these effects (*55*). Mutational studies further support the role of aromatic residues in driving phase separation, in particular through π-π stacking (*53*, *56*, *57*). Hydrophobic interactions, an important and related physicochemical driving force, may also play a role in intracellular condensation. Although typical condensed phases dissolve upon increasing temperature, which is associated with an upper critical solution temperature (UCST), hydrophobic interactions can manifest in lower critical solution temperature (LCST)—phase separation upon increasing temperature, as observed in vitro with several protein and model polypeptide systems (*49*, *53*, *58*, *59*).

Although in many cases the homotypic interactions among different IDRs drive phase separation, heterotypic interactions are also important. Both specific RNA/protein interactions and nonspecific interactions—for example, those associated with charged residues—are likely to play key roles, as we discuss further below. In synthetic systems, it has long been recognized that two types of oppositely charged polymers or colloids can first form complexes, which then undergo phase separation at high enough concentrations in a process referred to as complex coacervation (*60*). Evidence for this has recently been shown for the disordered intracellular domain of nephrin (NICD), whose net charge of –21 facilitates complex coacervation in vitro in the presence of positively supercharged green fluorescent proteins,

resulting in the formation of liquid-like droplets (42). Moreover, when overexpressed in HeLa cells, NICD forms liquid condensates within the nucleus. Consistent with complex coacervation but not with simple coacervation, the concentration outside of NICD condensates is not constant as the expression level increases. NICD presumably undergoes complex coacervation together with positively charged proteins in the nucleus to neutralize its charge for phase separation.

Highly multicomponent systems

The work described above has begun to shed light on the molecular-level rules important for the assembly of liquid-like organelles. But such condensates exhibit remarkably diverse composition; for example, there are dozens of proteins found in P granules (61) and stress granules (62), and mass spectrometry of purified nucleoli has identified many hundreds of enriched proteins (63). In all of these cases, only a small subset of proteins are necessary to promote in vitro liquid droplet formation (41, 64–68), but the larger set of components appear to act in concert to control phase separation in living cells. Moreover, RNP bodies typically contain not only proteins but also numerous RNAs and clearly represent highly multicomponent systems. How the droplet composition, biophysical properties, and protein phase behavior would be altered as the system incorporates more components is an outstanding question, which is only beginning to be addressed (Box 3).

The role of RNA

Recent work has begun to uncover a multitude of different effects of RNA on intracellular phase transitions. In many cases, RNA appears to promote phase separation. For example, polypyrimidine tract-binding protein (PTB), an RNA-binding protein with four RNA recognition motif (RRM) domains, undergoes liquid-liquid phase separation in the presence of RNA, independent of any IDRs involved (17), and the N-terminal fragment of hnRNPA1 containing two RRMs also shows similar behaviors (15). Moreover, the nucleolar protein nucleophosmin (NPM1/B23) assembles into a pentamer, which requires ribosomal RNA (rRNA) to form multivalent interactions that drive phase separation (64). In many cases, these proteins contain both RNA-binding motifs and IDRs (Fig. 2B) (35), and interactions with RNA promote liquid-liquid phase separation synergistically with IDR-IDR interactions. This manifests as a lowering of the saturation concentration for phase separation in the presence of RNA, an effect now seen with numerous proteins (15, 54, 66, 69, 70).

RNA can have other effects on the phase behavior of intracellular condensates. The saturating concentration of the P granule protein LAF-1 was found to be insensitive to the presence of short [50 nucleotide (nt)] polyadenylate [poly(A)] RNA, even while the physical properties of the droplets such as the internal viscosity are strongly affected (41). This arises from the way in which RNA selectively skews the right arm of the binodal (definition provided in Fig. 3), causing the protein concentration within droplets to markedly decrease, with an associated decrease in droplet viscosity (46). These effects of added RNA arise in part from RNA's impact on protein interaction strength, which is typically quantified through the dissociation constant or closely related second virial coefficient (71). Entanglement may also be relevant because longer poly(A) RNA (3000 nt) can have the opposite effect, increasing LAF-1 droplet viscosity (46); entanglement could also explain the finding that long RNA increases the viscosity of Whi3 droplets

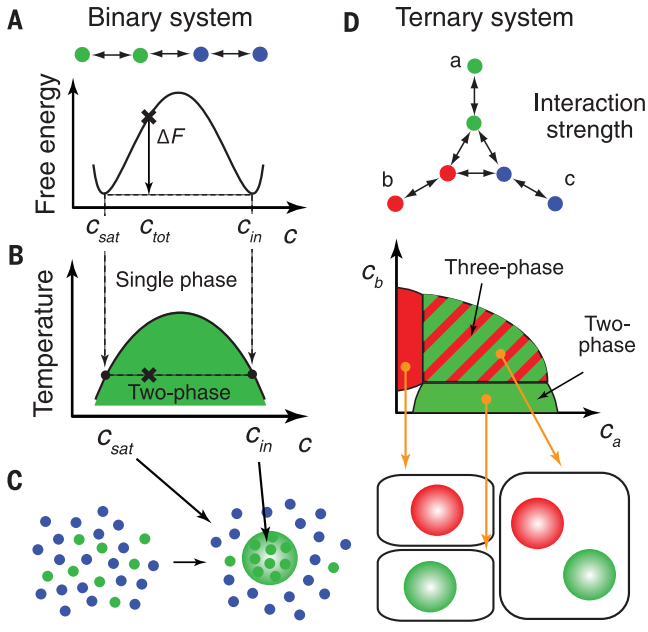

Fig. 3. Thermodynamic basis of phase separation. (**A**) Phase separation is a thermodynamic process in which a uniformly mixed system can lower its free energy by dividing into two or more phases with distinct compositions: for binary mixtures, C_{sat} and C_{in}. (**B**) The free-energy construction at different temperatures can give rise to a full phase diagram showing conditions under which phase separation occurs. The sharp phase boundary dividing single-phase and two-phase regions is called the binodal; the left arm of the binodal dictates C_{sat}, and the right arm dictates C_{in}. (**C**) In the binary mixture, phase separation leads to the formation of regions enriched with one component in equilibrium, with a surrounding region enriched with the other component. (**D**) In a ternary system, the shape of phase diagrams can differ depending on interactions between components. One possibility is a three-phase region, as well as distinct two-phase regions in the phase space. In the three-phase region, two distinct types of liquid droplets can coexist.

Box 3. Theoretical considerations in multicomponent phase separation.

An important theoretical framework for understanding phase separation is the regular solution model [or the closely related Flory Huggins model for polymers (30)]. This model considers molecules interacting on a lattice. The number of possible distinct ways of placing the molecules on the lattice dictates the entropy of mixing. The enthalpic contribution to the free energy arises from assigning pairwise interaction energies between neighboring molecules on the lattice and approximating their contribution by statistically averaging the effect of all such interactions into a much simpler "mean-field" term. This approximation dramatically reduces the complexity of the problem while still capturing key behaviors of phase-separating systems. The regular solution as well as more advanced theoretical frameworks—for example, considering charge patterning effects—have been used to model RNA/protein phase behavior (16, 70, 176). The regular solution model can be extended to larger numbers of components—for example, in the so-called ternary solution, which includes additional terms for entropy and interaction energies. The ternary regular solution model has been successful in describing P granule dynamics (66, 133) and the dynamics of engineered IDP constructs (96).

The ternary regular solution model provides useful insights in understanding the complex phase behaviors possible in multicomponent systems (177, 178). The exact shape of the phase diagram, including the presence of coexisting phases, is determined by intermolecular interactions between constituting components. In general, the system can have two-phase regions along each composition axis. Under a case in which these two-phase regions from each component are large enough to meet in the middle of a phase diagram, there can be two distinct possibilities for the phase coexistence. Again, depending on the relative magnitudes of intermolecular interaction strengths, the system can have either a three-phase coexistence region or a single interconnected two-phase region (Fig. 3). Theoretical approaches for expanding our understanding of phase behavior in the highly complex intracellular environment have begun (179, 180), but more work is needed.

(54). However, in the latter case, CLN3 and BNI1 RNA exhibit specific Whi3-binding sequences as well as secondary structure. As we discuss further below, the nature of these RNA/protein interactions has important biological implications—for example, in the patterning of developing embryos and in nucleating various nuclear condensates (72). Moreover, recent work supports the idea that the phase behavior of RNA itself is important and could play a role in neurodegenerative diseases associated with trinucleotide RNA repeats (73).

Multiphase immiscibility

The concept that many intracellular condensates represent phase-separated liquid states of RNA and protein may seem at odds with the fact that these structures do not always appear to be homogeneous spherical droplets. Instead, they can assume nonspherical shapes and exhibit inhomogeneities over "mesoscopic" length scales, much larger than characteristic molecular dimensions. One of the most well-known examples is the nucleolus, the largest nuclear body, which exhibits a core-shell architecture thought to be important for sequential processing of nascent rRNA transcripts (74). A similar core-shell structure is observed in stress granules (62), and RNP bodies such as processing bodies and P granules have been reported to exhibit some degree of internal structuring (75–77).

Recent work has provided evidence that the core-shell architecture of nucleoli self-assembles through the immiscibility (nonmixing) of multiple liquid phases (Fig. 4) (65). Key components found in nucleolar subcompartments exhibit rapid molecular dynamics as seen in fluorescence recovery after photobleaching (FRAP). Moreover, a number of these proteins are capable of phase separating in vitro. As discussed above, the nucleolar "shell" protein NPM1 forms liquid droplets in the presence of rRNA (64), whereas the "core" protein fibrillarin also undergoes liquid-liquid phase separation in vitro, which is also sensitive to rRNA (70). Remarkably, when these two different types of purified protein droplets are combined, they do not fuse with one another but remain as distinct, immiscible phases (65). Moreover, fibrillarin-rich droplets are engulfed by NPM1-rich droplets, similar to the core-shell organization of the nucleolus in vivo. Although the molecular determinants of protein phase immiscibility are still poorly understood, systematic efforts are under way (78).

Multiphase coexistence is well known in nonliving systems and can be demonstrated with simple mixtures of organic solvents (79). Depending on relative droplet surface tension, this can give rise to structured droplets (80); for example, droplets with a high surface tension will be engulfed by the lower surface tension droplet (Fig. 4A). This is analogous to the burying of energetically costly hydrophobic residues within a folded protein to minimize its interaction with water. A similar principle has been suggested to explain the internalization of nonmixing tissues (81). These findings could help explain the core-shell structures observed in several intracellular condensates, including paraspeckles (82), PML bodies (83), and stress granules (62), although in the latter case, an ordered assembly model has been proposed (84).

Beyond such core-shell architectures, these findings have broader implications for other structures. For example, high interdroplet surface tensions likely explain why some neighboring condensates do not interact at all but instead remain as physically separate droplets (Fig. 4A, case iii). However, in other cases the surface tensions can be such that two distinct phases are able to partially interact, but without full engulfment (Fig. 4A, case iv). This may underlie observations of partial interaction among P-bodies and stress granules (85), as well as Cajal bodies/HLBs and snurposomes (Fig. 4D) (86). Moreover, the nucleolus develops lobulated perinucleolar caps upon ribosomal DNA (rDNA) transcriptional inhibition (Fig. 4C) (87), which may reflect changes in the relative surface tensions. Proteins have been identified that localize to condensate surfaces (13, 88) and could serve to modulate surface tension, which is consistent with a recent study showing that the charged protein Ki-67 functions as a chromosome surfactant (89).

Liquid metastability and cell pathology

As discussed above, the condensed liquid states of proteins are well known in purified protein solutions, and so too are other, more complex phase behaviors. Structural biologists have long observed that protein droplets will sometimes exhibit "metastability"; they only exist as stable droplets for some time before nucleating protein crystals (90, 91). Hemoglobin solutions have also been observed to form phase-separated liquid droplets that are similarly metastable, which could be important for sickle cell anemia (92). A number of experimental and theoretical studies have provided key insights, particularly highlighting the role of molecular interaction length scale in dictating the stability of the liquid-liquid coexistence region of the phase diagram (93, 94).

Several lines of evidence indicate that condensates exhibit liquid metastability. First, FUS, hnRNPA1, and other ALS-associated RNP body proteins can form hydrogels composed of amyloid-like fibers in vitro at high concentrations and/or aged samples (37, 51, 95). It is now clear that FUS, hnRNPA1, Whi3, fibrillarin, and other purified IDR-containing protein solutions will initially undergo liquid-liquid phase separation, but over time the resulting droplets exhibit more solid-like features (Fig. 5) (14, 15, 54, 57, 65, 69). FUS, in particular, forms spikelike fibers that emerge from droplets that have been sitting on a coverslip for several hours (Fig. 5B) (14); fibrous structures are also seen to nucleate within other ALS-associated protein droplets (15, 51, 52, 69).

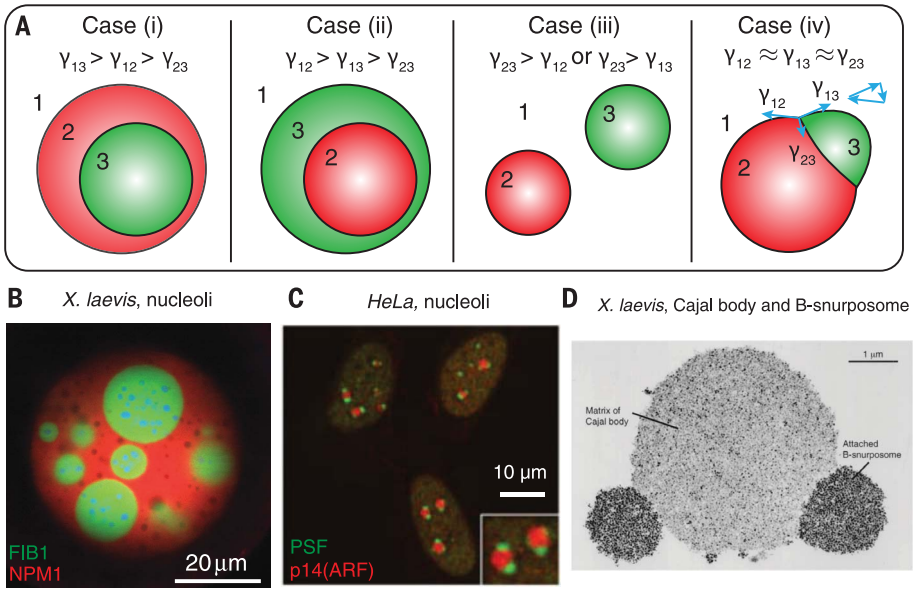

Fig. 4. Surface tension and multiphase droplet architecture. (**A**) The relative surface tensions among the different possible interfaces (γ_{ij}) dictate the droplet architecture. Minimizing the free energy of the system requires minimizing energetically costly interfaces. For example, in case i the costly 1–3 interface (γ_{13} large) is avoided by phase 2 enveloping phase 3, whereas case ii achieves the opposite. For case iii, the interface between the two droplets is costly (high γ_{23}), and thus, droplets do not contact one another. When relative energetic costs are comparable, all three phases can have shared interfaces, as shown in case iv. Surface tensions from three interfaces are balanced, forming Neumann's triangle (161). (**B**) The multiphase nucleolus after actin disruption in *X. laevis* (65). (**C**) Transcription inhibition leads to reorganization of nucleolar architecture, forming perinucleolar caps bound to nucleolar bodies (87). (**D**) Electron micrograph showing *X. laevis* Cajal body with attached B-snurposomes (86).

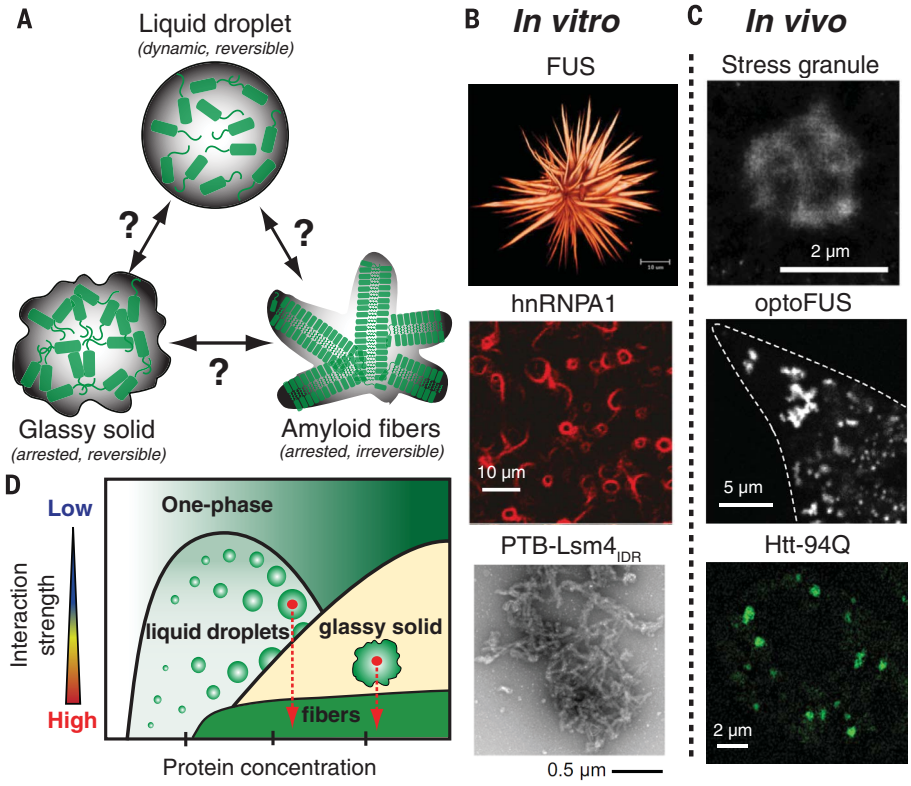

Fig. 5. Liquid phase metastability is linked to solid-like condensed states. (**A**) Depending on conditions, a protein can condense into different high-order structures such as liquids, glassy solids, or amyloid fibers. Molecular dynamics, internal organization, and reversibility of these condensed phases are distinct. (**B**) In vitro liquid droplets that formed from several IDPs, especially those with prion-like domains, often undergo a liquid-to-solid transition to form fibrous structures (*14*, *15*, *69*). (**C**) (Top) In cells, native RNP bodies often exhibit solid-like characters (*62*). (Middle) The glassy solid state can be induced in living cells by using optogenetic approaches (*96*). (Bottom) The expression of disease-inducing mutant proteins often leads to aggregate formation, as shown for the Huntingtin protein fragment with poly(Q) expansion (*162*). (**D**) Depending on where the cell is located in this schematic phase diagram, intracellular condensates with distinct material properties can be assembled. Deep within the two-phase region, dynamically arrested glassy solids appear to form. Liquid and/or glassy solids may mature into the fiber state.

It is noteworthy that similar "sea urchin" morphologies are also seen in solutions of lysozyme, a well-folded globular protein, (*21*), although the transitions within the IDP droplets appear to correlate with sequence features associated with pathology in patients. However, disease-related mutations do not necessarily alter liquid-liquid phase separation, suggesting that although phase separation and amyloid formation may be linked, they can be distinct processes (*15*, *69*).

The majority of these studies have been performed with purified proteins, but the situation in living cells is less clear. One recent study has probed phase transitions in living cells by using optogenetic protein constructs fused to various IDRs, including FUS, DDX4, and hnRNPA1 (*96*). Upon blue light activation, the constructs cluster into cytoplasmic and nuclear "optoDroplets," but only above a saturating threshold concentration, which is consistent with their assembly representing light-activated phase separation. When the system is only moderately supersaturated above this threshold, FUS optoDroplets are spherical and exhibit liquid-like behaviors, including coalescence and full FRAP recovery, similar to endogenous liquid condensates. However, when light is used to drive the system deeper into the two-phase region, the resulting clusters become more solid-like, with irregular morphology and incomplete FRAP recovery. These solid-like assemblies appear as the phase separation proceeds, which is distinct from amyloid-containing hydrogels requiring a long incubation at high protein concentration (*37*).

These findings echo previous work in model protein and colloidal systems, in which deep within the two-phase region there is often a "glass" or "gel" boundary at which strongly interacting particles can no longer sample different configurations (*21*, *97*–*99*); at the molecular level, such glassy states are relatively unstructured and indistinguishable from conventional liquids, even while their fluid dynamics are entirely arrested (*100*, *101*). The concept of dynamically arrested condensates would be consistent with microrheology studies that have shown that in vitro droplets of Whi3 and LAF-1 have malleable and composition-dependent material properties (*41*, *54*). Whether native condensates exhibit liquid-like properties, versus more solid-like properties, thus likely depends on the degree to which the cell is supersaturated—how deeply the system is driven into the two-phase region (Fig. 5).

A key question concerns the reversibility of these condensed states, which is relevant for biological function and the diseases associated with many of the component proteins. A number of studies found that in vitro droplets assembled from purified IDP constructs can be initially disassembled upon changes in temperature or the concentration of protein or salt (*16*, *69*). However, over time their reversibility is compromised, and small fibrous deposits often remain (*54*, *69*). For example, upon cycling the ALS-related proteins hnRNPA1 and FUS through multiple rounds of droplet assembly and disassembly, they begin to show increasing resistance to disassembly (*15*, *51*). However, the situation within living cells may be different; whereas assembly of liquid-like FUS optoDroplets is fully reversible even after five or more activation cycles, the more deeply supersaturated solid-like structures begin to resist disassembly after several cycles, resulting in aggregates that persist in cells for hours (*96*). Thus, within living cells, it is unclear whether the liquid states are inherently prone to pathological conversions or whether it is an unstructured solid-like state—associated with particularly high concentrations of dynamically arrested IDRs—that slowly nucleates irreversible amyloid fibers.

Given the link between material properties and pathological conditions, cells have likely evolved mechanisms to monitor and control the fluidity of these structures. This is particularly interesting when considering condensates that exhibit more solid-like material properties (nonspherical, nonfusing, and little FRAP recovery). The solid-like condensates may assemble either through arrested liquid-liquid phase separation, leading to the glass/gel state, or formation of amyloid-like fibers. For example, the Balbiani body is a solid-like structure held together by a matrix of amyloid-like fibers (*102*). Yeast stress granules also exhibit solid-like features, yet possibly without involving amyloid-like assembly (*103*), and mammalian stress granules contain solid-like cores, surrounded by a more liquid-like phase (*62*, *84*). These solid-like material states are likely affected by protein and RNA quality control machinery, including molecular chaperones, adenosine 5′-triphosphate (ATP)–dependent RNA helicases, and other adenosine triphosphatases (ATPases) (*62*, *104*–*109*). Many RNP bodies are enriched in RNA helicases, as well as protein chaperones, including HSP104 in yeast and the HSP40/70 system in metazoa (*62*, *110*, *111*). Consistent with this, the viscoelasticity of nucleoli, particularly the fibrillarin-rich nucleolar subcompartment, exhibits a strong ATP dependence (*13*, *65*), whereas stress granule component dynamics decrease upon ATP depletion (*62*). The detailed molecular mechanisms by which these

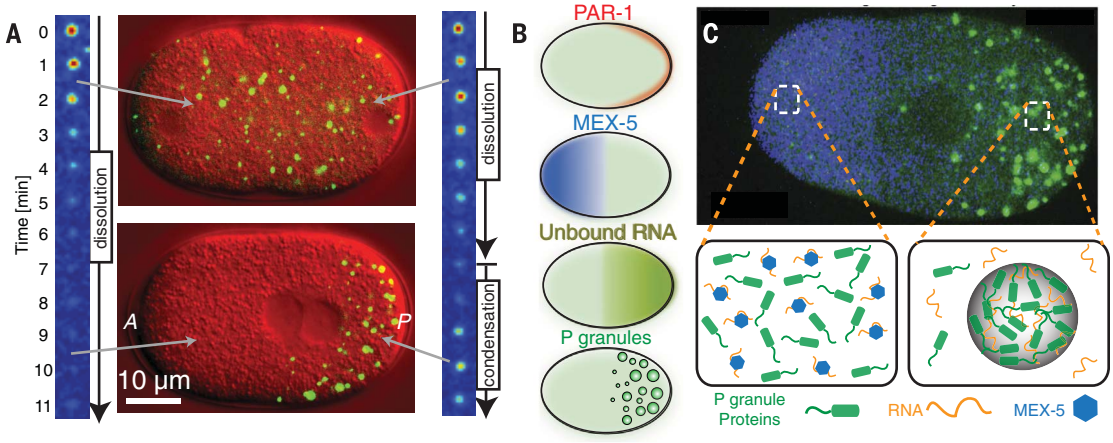

Fig. 6. P granules exemplify phase separation coupled to a reaction-diffusion system. (**A**) In the single-cell stage of *C. elegans* embryos, liquid-phase P granules undergo spatial segregation to the posterior side of the cell through controlled dissolution and condensation (*12*). (**B**) The localization control of P granule segregation involves a set of interlinked reaction-diffusion systems that pattern the embryo cytoplasm, initiated by the asymmetric distribution of PAR-1 and other polarity proteins. The MEX-5 gradient, formed by localized activity of PAR-1, appears to drive the accumulation of free RNA in the posterior side. This in turn facilitates preferential posterior liquid-liquid phase separation of P granule components. (**C**) A molecular-level schematic of how the MEX-5 gradient patterns P granule distributions via an RNA competition mechanism (*66*).

ATPases regulate condensates is still largely unclear but may involve localized internal forces that control fluidity, analogous to the tunability of concentrated actin solutions by myosin motor activity (*112*). However, global ATP depletion influences material states of the entire cytoplasm (*113*, *114*), potentially through its functions as a solubilizing intracellular hydrotrope (*115*), further underscoring the multifaceted role of ATP in intracellular fluidization. Combined efforts with targeted RNA interference or mutations specifically abrogating ATPase activity can help elucidate how material states are monitored and controlled and may suggest disease intervention strategies based on modulating condensate phase behavior.

Controlling condensation through biological activity

The apparent metastability of liquid condensates underscores the fact that living cells are far from thermodynamic equilibrium, and in many cases these structures likely do not represent equilibrium thermodynamic phases. In addition to the increasing number of studies reporting ATP-dependent properties, it is also clear that many other dynamic reactions control the phase behavior of condensates. The nucleolus presents one interesting case: As discussed above, in vitro droplets formed from nucleolar RNA-binding proteins exhibit a thermodynamic preference for RNA, causing droplets to preferentially grow at transcriptionally active rDNA loci (*70*, *116*). Moreover, inhibiting transcription after nucleolar assembly causes dramatic changes in composition and structure (*87*). Thus, nucleolar assembly may occur largely through thermodynamic driving forces, but these are modulated by localized RNA transcription and likely other nonequilibrium features intrinsic to living cells. Indeed, measurements of the temperature dependence of nucleolar assembly in vivo suggest that although some nucleolar proteins condense at lower temperatures, others show the opposite behavior (*117*). Although interpretation may be complicated by the possibility of both UCST and LCST behavior, as well as caveats associated with phase separation versus molecular partitioning, taken together these studies underscore the additional layers of active biological control, beyond equilibrium thermodynamic driving forces.

The findings of RNA promoting nucleolar condensation are consistent with previous work showing that concentrating RNA locally or ectopic transcription of RNA leads to spontaneous formation of related nuclear bodies, including paraspeckles, Histone locus bodies, Cajal bodies, and nuclear stress bodies (*118*, *119*). These examples suggest that there are powerful mechanisms that can serve as a feedback for condensation, creating "on-demand" droplets promoted by the presence of unprocessed transcripts. On-demand assembly may also take place with stress granules and DNA damage foci, which respond to the presence of environmental stress (*4*, *120*). Reaction-controlled assembly also appears to play a role in the centrosome, where cycles of reactivity associated with localized kinase activity drive centrosomal proteins to condense around a single centriole, preventing the formation of multiple centrosomes that could promote mitotic defects (*121*, *122*).

Given the important role of intermolecular interactions in the phase behavior as discussed above, dynamic posttranslational modifications (PTMs) of polypeptide chains are likely to be a key regulatory mechanism that cells use to control compositions, dynamics, and stability of condensates. For example, arginine methylation leads to a 10-fold increase in the concentration required for in vitro DDX4 liquid droplet formation because of perturbed cation-π interactions (*16*). Phosphorylation similarly affects the assembly of in vitro peptide-RNA liquid droplets (*123*). Inside cells, altered interaction strengths of phosphorylated proteins can manifest in several distinct ways, including increased internal dynamics (*124*), release from condensates (*125*, *126*), and dissolution of the entire condensate structure (*25*, *76*, *127*, *128*). Phosphorylation can conversely stimulate phase separation when specific protein-protein interactions are involved, as exemplified by phosphorylated tyrosine-SH2 binding (*17*). PTMs can also act in seeding condensation, as shown in a highly negatively charged poly (adenosine 5′-diphosphate–ribose) (PAR) (*120*), likely in a manner similar to that by which RNA nucleates liquid condensates.

Historically, the power of systems of reacting and diffusing molecules was first highlighted in 1952 by Alan Turing, who introduced a mathematical framework explaining how coupled reaction diffusion equations can explain the spontaneous generation of patterns of biomolecules (*129*); this basic mechanism is now understood to occur in systems as diverse as bacterial Min proteins, skin pigmentation, and embryonic patterning (*130*, *131*). Theoretical frameworks for reaction-diffusion have been linked to phase separation dynamics to describe nonliving systems (*132*). However, with only a few exceptions (*66*, *70*, *96*, *133*), little work has been done combining experimental and theoretical investigations of reaction-controlled intracellular condensation. In the one-cell *C. elegans* embryo, a reaction-diffusion network of interactions involving polarity proteins, particularly the kinase PAR-1, results in a polarized embryo cortex and cytoplasm (Fig. 6). Localized kinase activity in turn establishes a MEX-5 gradient across the embryo (*134*). Because of differences in affinity of polarity proteins for mRNA, the MEX-5 gradient gives rise to localized phase separation at the posterior side of the embryo, providing a fascinating example of the interplay between a reaction-diffusion patterning system and RNA-facilitated phase separation (Fig. 6) (*12*, *66*, *67*, *133*).

Functional importance of intracellular phase separation

A key question concerns the functional consequences of liquid states in healthy cells. Conceptually, three categories can be distinguished as being important for cellular physiology (Fig. 7); yet it should be noted that these categories are not mutually exclusive, and single droplets could

exhibit all three functions with varying degrees. At present, our understanding of condensate function lags behind the rapidly developing elucidation of molecular assembly mechanisms, underscoring the need for future work.

Reaction crucible

Chemical reaction rates depend on concentrations of reactants. Concentrating a specific set of molecules into the condensed state may facilitate efficient cellular reactions between weakly interacting molecules (Fig. 7). Moreover, the liquid-like nature of many condensates allows for dynamic exchange of reactants and products, as exemplified by FRAP experiments that demonstrate typically rapid fluorescence recovery. The functional link between phase separation and increased reaction rates has been highlighted in the multivalent SH3/PRM system. The actin nucleation promoting factor neural Wiskott-Aldrich syndrome protein (N-WASP) contains six PRMs, which can bind SH3 domains of NCK and induce phase separation. Multivalent SH3/PRM interactions promote phase separation, concentrating actin nucleation factors and resulting in local actin polymerization within droplets (*17*, *135*). Recent work shows that these SH3/PRM–driven phase transitions can also promote signaling outputs both in an in vitro reconstituted system and in living cells (*136*). During T cell receptor (TCR) signaling, signaling components become activated by a series of phosphorylation reactions, leading to the formation of micrometer- or submicrometer-sized clusters (*137*). In the reconstituted system, linker for activation of T cells (LAT), a critical adaptor protein for TCR signaling, undergoes liquid-liquid phase separation that is highly dependent on the level of available multivalency. Higher multivalency also leads to stronger activation of mitogen-activated protein kinase (MAPK) signaling in Jurkat T cells, supporting the idea of condensate-enhanced signaling.

RNP bodies may similarly function to concentrate reactants and thereby enhance reaction rates, as has been suggested for nucleoli, Cajal bodies, and splicing speckles. It has also long been speculated that such phase-separated droplets may have served as protocellular reaction crucibles (*138*, *139*); recent theoretical work suggests that phase-separated droplets may have even been able to grow and divide through reaction cycles (*140*). The possibility for such droplet protocells to concentrate RNA is particularly interesting, given the likely central role of RNA in early life. A model experimental system used phase separation of polyethylene glycol (PEG) and dextran, which forms droplets that are able to concentrate ribozymes, RNA enzymes similar to those that may have been key for the origin of life. PEG/dextran droplets were able to speed up ribozyme reaction rates by nearly two orders of magnitude (*141*).

Sequestration

Molecular condensation may function to sequester factors not required for cellular needs and thereby prevent any off-target effects (Fig. 7). The nucleolus functions in part as a reaction crucible for rRNA biogenesis, but it has long been thought to have additional functional roles, particularly in the cell cycle and stress-dependent sequestration of key signaling molecules (*74*). Cytoplasmic stress granules provide another rich example of sequestration, functioning as microcompartments for concentrating stalled translation complexes under cellular stress conditions. Among numerous factors enriched in stress granules are components of signaling pathways, including target of rapamycin complex 1 (TORC1) (*110*). Sequestration of TORC1 into the stress granule represses TORC1 signaling (*124*, *142*), highlighting a link between the cytoplasmic compartmentalization and cellular signaling. The kinase DYRK3 localizes to stress granules and regulates their dissolution (*124*). Transient expression of DYRK3 in HeLa cells leads to liquid-liquid phase separation to form a condensed liquid phase of DYRK3 in the cytoplasm. A kinase-deficient version of DYRK3, instead, forms more solid-like aggregates, indicating that the kinase activity of DYRK3 can affect the material properties of stress granules. The material properties of such condensates are intimately linked to their molecular dynamics, which in turn can affect their ability to sequester relevant factors. Future work will be necessary to quantify the extent of sequestration and to shed more light on the coupling between the tunable material properties of condensates and their multifaceted biological functions.

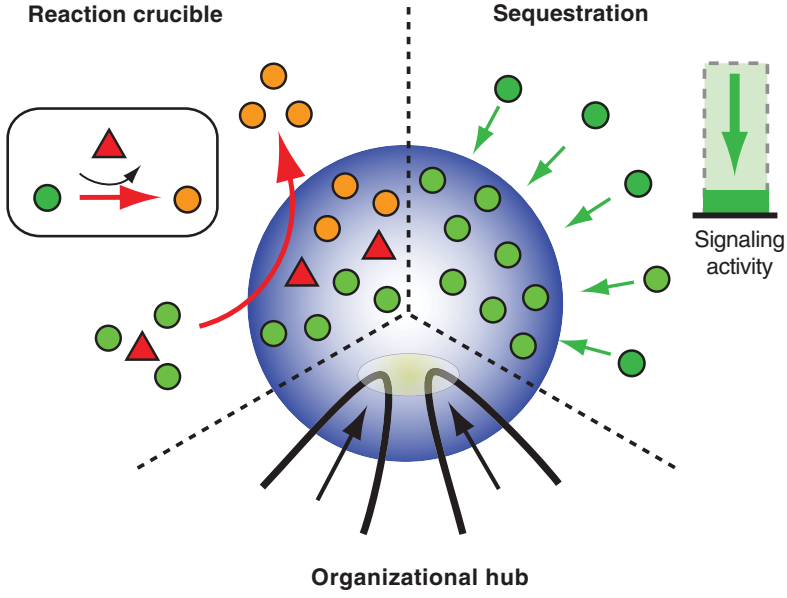

Fig. 7. Functional roles of intracellular phase transition. Three functional categories are shown by which intracellular condensates play a role in cell physiology. (**Left**) Concentrating a specific set of molecules can enhance biological reactions, which is further facilitated by the dynamic molecular nature of liquid phases. (**Right**) Sequestration of key signaling complexes into condensates can coordinate response to environmental stress and cell signaling. (**Bottom**) Condensates can also function as an organizational hub. For example, the localization of nuclear bodies and chromosome organization are often coupled.

Organizational hub

Liquid-liquid phase separation and the resulting condensates also appear to be exploited by cells to organize their internal space. One interesting recent example suggests that liquid phase condensation may play an important role in organizing spindle assembly. BuGZ is a *Xenopus* microtubule-binding protein that is predicted to be mostly disordered and was found to undergo liquid-liquid phase separation in vitro. The resulting droplets are capable of bundling and concentrating tubulin and may play an analogous role in organizing the spindle in living cells (*53*). Another recent study suggests that the forces arising from the surface tension of membrane-associated condensates contribute to endocytosis by promoting membrane invagination (*143*), echoing the paradigm of multiphase droplet structuring through surface tension effects.

Liquid phase condensation appears to play similar organization roles within the nucleus, whose internal organization is entirely achieved in the absence of membrane-bound subcompartments (Fig. 7) (*3*). It has become abundantly clear over the past decade that chromosomes and associated nuclear bodies are not randomly distributed in the nucleus (*144*), and nuclear architecture is intimately associated with dynamic gene regulation (*144*, *145*). Repressed genes often cluster into large compact states known as heterochromatin, and recent work demonstrates that heterochromatin in early *Drosophila* embryos, observed with heterochromatin protein 1a (HP1a), exhibits signatures of liquid droplets including fusion and dynamic molecular exchange (*146*).

Closely related in vitro work with human HP1α provides additional molecular-level insights into the role of liquid-phase protein/DNA condensates in gene silencing (*147*). This work is reminiscent of studies of mitotic chromatin mechanics, in which partial restriction enzyme digestion causes the rounding of chromatin droplets (*148*). In all of these cases, forces associated with droplet surface tension likely play a key structuring role; however, more fundamental biophysical studies on the impact of condensates on DNA sequestration and organization are needed (*149*).

The nucleolus is the largest nuclear body and presents another interesting example of a condensate strongly affecting genome organization, with ~4% of the mammalian genome in nucleolus-associated chromatin domains (NADs) (*150*). Moreover, several genomic loci for small nuclear RNA (snRNA) or small nucleolar RNA (snoRNA) have been shown to associate with Cajal bodies, and disassembly of Cajal bodies leads to disruption of these gene clusters and suppressed expression of related genes (*151*). Although their material states and assembly are still poorly understood, transcription factories as well as repressive polycomb group (PcG) bodies also seem to harbor multiple genomic loci for coregulation of several related genes, which is crucial for proper developmental processes (*152*, *153*). How highly localized phase separation coupled to local active processes, such as transcription (*154*), may serve to structure nuclear contents on 10- to 100-nm spatial scales represents a major open question; finite size effects on phase transitions will become increasingly important on these scales (*29*) but must be carefully considered in this rich biological context.

Conclusions

Concentrated states of RNA, protein, and DNA are a central aspect of intracellular organization. It has become increasingly clear that a large variety of these condensates form via liquid-liquid phase separation, resulting in liquid-like states of intracellular matter. The list of condensates thought to assemble through liquid-liquid phase separation now includes many RNP bodies such as P granules (*12*), nucleoli (*13*), various signaling clusters (*17*, *136*), and numerous other related structures. Liquid-liquid phase separation may also play a key role in a more diverse set of structures, including postsynaptic density (*155*), the synaptonemal complex (*156*), and the mitotic spindle (*53*). Thus, condensation of intracellular liquids can drive assembly of structures far richer than homogeneous droplets, ranging from multilayered structures such as the nucleolus and stress granules to liquid-crystalline assemblies such as the spindle (*157*, *158*) and actin condensates (*159*). It will be important to understand how sequence-encoded information can drive complex multiphase coexistence and the physicochemical properties of these diverse and richly structured condensates. Of particular biomedical importance is the need to understand how metastable transitions of liquid condensates into more solid-like states could underlie devastating protein aggregation diseases. More work is also needed to uncover regulatory mechanisms used by cells to control biomolecular phase behavior, orchestrate the assembly of various physiological structures, and prevent pathological conversions. In all cases, the molecular components of these structures appear to interface closely with the inherently nonequilibrium features of the cell. Understanding this interplay between equilibrium thermodynamic driving forces and nonequilibrium activity is a central challenge and will be critical for elucidating how condensed liquid states of intracellular matter contribute to both cell physiology and disease.

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Liquid phase condensation in cell physiology and disease

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Phase separation and cellular organization

Cells are compartmentalized to allow distinct processes to occur in membrane-delimited organelles. But similar spatial restriction of cellular components in membrane-less intracellular assemblies or condensates also appears to occur—much like oil droplets in water. These compartments contribute to multiple biological processes and regulatory mechanisms. Shin and Brangwynne review the protein-protein and protein-RNA interactions that result in formation of these structures. They explain known and potential functions of such structures in a range of examples, from signaling and local control of biochemical reactants to spatial segregation. In disease, such aggregation may go awry and contribute to neurodegenerative syndromes associated with inappropriate protein aggregation.

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